

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Steven M. RUBEN

Appl. No.: 10/662,429

Filed: September 16, 2003

For: **Apoptosis Inducing Molecule I**

Confirmation No.: 2663

Art Unit: 1644

Examiner: HUYNH, PHUONG N.

Atty. Docket: 1488.1890003/EJH/SAC

**Declaration of Kong B. Tan**  
**Ruben Exhibit #25**

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Ruben EXHIBIT #25

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Paper No. \_\_\_\_\_

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UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES  
(Administrative Patent Judge Sally Gardner Lane)

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STEVEN M. RUBEN

Junior Party,  
(Application No. 08/816,981),

v.

STEVEN R. WILEY  
and RAYMOND G. GOODWIN

Senior Party,  
(Patent No. 5,763,223).

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Patent Interference No. 105,077

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DECLARATION OF KONG B. TAN

Ruben EXHIBIT 2025  
Ruben v. Wiley et al.  
Interference No. 105,077  
RX 2025

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DECLARATION OF KONG B. TAN

I, Kong B. Tan, declare and state as follows:

1. I am employed by GlaxoSmithKline (formerly SmithKline Beecham or "SB") and acted in a scientific role as a Senior Investigator at SB during the time periods discussed below.

I have been asked by patent counsel to Human Genome Sciences ("HGS") to describe my activities relating to AIM-I.

2. I conducted a series of RNA blot ("Northern analysis") experiments to reveal and compare expression patterns of AIM-I and several other novel TNF/TNFR family members. I documented these experiments in the notebook pages referred to herein. I alternatively referred to AIM-I as "TL2" and "TNLF1." In August 1995, I carried out a first Northern analysis of AIM-I expression together with several other TNF/TNFR family members ( RE26 ).

Beginning on August 16, 1995, Jurkat cells were treated with 25 nM TPA for 24 hours. On August 18, 1995, RNA from the Jurkat cells as well as from brain, heart, lung, thymus, spleen, liver, kidney, small intestine, and prostate was run on a set of three electrophoretic gels, each with a duplicate set of RNA samples, to make six sets of RNA samples ( RE26 ). The gels were blotted on August 18, 1995. On August 21, 1995, a blot of each of the six individual RNA samples was probed with a specific TNF ligand or TNF receptor probe, including an AIM-I probe (blot 3, labeled "343-413412") ( RE26 ). The autoradiogram is dated August 18, 1995, the date of the RNA blotting. Blot hybridization and development was begun on August 21, 1995 (including a one hour pre-hybridization, a fifteen hour overnight hybridization, and exposure to autoradiography film) and continued at least until August 25, 1995 before film development. My usual practice for Northern blots at that time was to exposed an autoradiogram

for about three to five days. Accordingly, this first Northern analysis entailed at least eight days of continuous activity (*i.e.* at least August 16-18 and 21-25, 1995), and more likely nine to eleven days of continuous activity.

3. I performed a second Northern analysis of AIM-1 in mid-September of 1995 ( RE27 ). For this experiment, a set of two gels, each with duplicate RNA samples of various cell lines (*i.e.*, MG63, TF274, KG1a, KG1, NL60, THP1 CD19+, CD4+, BM105, MCF7, Molt 3) were electrophoresed on September 20, 1995 and blotted the same day ( RE27 ). Each of the four resultant blots was hybridized for 15-20 hours with various probes including an AIM-I probe (blot 2, labeled "ATG 343/TNF 413412") ( RE27 ). The blots were then exposed to autoradiography film for five days. Accordingly, this second Northern analysis entailed at least six days of activity beginning September 20, 1995, not accounting for additional time preparing RNA samples and probes, which the experiment necessarily also entailed (*i.e.* at least September 20-26, 1995).

4. I performed a third Northern analysis prior to the October 18, 1995 joint HGS/SB meeting ( RE28 ). RNA samples from a combination of cell lines and tissues (*i.e.* Raji, Raji HN2, REH, CD8+, rat BM, rat thymus, rat spleen, rat heart, rat lung, rat kidney, rat small intestine) were electrophoresed on September 28, 1995, blotted and hybridized with various probes including AIM-I ( RE28 ). In accordance with my usual practice, this experiment likely entailed a full day of electrophoresis and blotting, a full day of hybridization, and at least three days of autoradiography. The autoradiogram dated October 2, 1995 shows the results for AIM-I (labeled "343 TL2") in the upper right-hand corner ( RE28 ). Accordingly, this third Northern analysis entailed at least five days of continuous activity (*i.e.*, at least September 28-October 2, 1995).

5. I performed a fourth Northern analysis employing treatment of six different cell types (*i.e.*, Jurkat, HL60, U937, THP1, KG1a and PLB) with either TPA or DMSO beginning on September 29, 1995 ( RE29 ). The cultured cells were fed daily prior to the three-day (70 hour) treatment. On October 2, 1995, cells were harvested and mRNA was prepared. On October 6, 1995, a second three-day (70 hour) treatment was begun with KG1a and PLB cells ( RE29 ). RNA samples were electrophoresed on October 12, 1995, blotted, and hybridized to probes ( RE29 ). In accordance with my standard practice at that time, this experiment entailed a full day of gel electrophoresis and blotting, a full day of hybridization, and several days of autoradiography. Accordingly, this fourth Northern analysis, beginning September 29, 1995, entailed about two weeks of continuous activity, including, at the very least, several days of cell culture maintenance and preparation prior to treatment, three days of a first cell treatment, three days of a second cell treatment, and at least five days of electrophoresis, blotting, probing, and autoradiography (*i.e.*, at least September 29-October 13, 1995).

6. I presented the results of these four Northern analyses at the October 18, 1995 HGS/SB meeting and provided RNA expression summary tables for inclusion in the meeting minutes.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-captioned application or any patent issuing thereon.

Date June 21, 2004

Kong B. Tan

